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Microalgal based bioactive compound: A novel source for antimicrobial resistance

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Abstract

Microalgae are the photosynthetic organisms, which are present in ocean waters and littoral environments. It is also known as phytoplankton a. Secondary metabolites are compounds that can be separated and purified from microalgae, plants, bacteria, and other organisms with the capacity for biological activity are known as bioactive chemicals. Terpenoids, steroids, phenolic compounds, alkenes, and phlorotannin are among the bioactive substances found in algae that have antibacterial properties. Numerous substances found in microalgae have the ability to inhibit bacteria and other microorganisms and serve as antibacterial agents. One of the primary emphasis areas for developing novel antimicrobial agents is marine microalgae. Using a variety of extraction methods and antimicrobial tests, many microalgae were assessed for their ability to defend towards gram-positive as well as gram-negative bacteria, particularly food and plant diseases. The antibacterial activity was verified using spot-on-lawn and disc diffusion tests. The extracts' efficacy was assessed by measuring their minimum inhibition concentrations. Strong inhibitory action was demonstrated by three microalgae, specifically *Tetraselmis suecica*, *Chaetoceros muelleri*, and *Isochrysis galbana,* against gram-positive bacteria. Following their selection for extra purification and investigation, these micro algae species were identified as compounds. Utilizing a combination of various chromatography methods, such as high-performance liquid chromatography, or HPLC, and gas chromatography–mass spectrometry (GC–MS), docosahexaenoic acid (DHA), Linoleic acid, oleic acid and eicosapentaenoic acid (EPA) are the main substances that were found and isolated in the extracts. These substances have the ability to stop gram-positive bacteria from growing. This suggests that microalgae and their antibacterial components might be used as bio control agents to prevent plant and food diseases.

Keywords: Microalgae; Bioactive Substances; Antimicrobial Activities; Natural Substances

1. Introduction

Since antibiotic are losing effectiveness at shocking rates due to the emergence of antibiotic-resistant germs, bacteria have become more resistant to them in recent decades, which has become a serious public health concern. Among the biggest healthcare concerns in the world today is the treatment of infectious diseases, particularly those brought on by resistant microorganisms. Major bacterial species have developed antibiotic resistances (Rossolini, 2014).

To address this, attention has turned to alternative sources such as marine life, which provides a rich and diverse array of novel, biologically active compounds. A number of chemically unique metabolites with different biological activity among marine origins, such as those from microalgae, have been isolated and developed as food and pharmaceutical products .The use of microalgae as an alternative source of antibiotics and preservatives has attracted similar attention. Eukaryotic and prokaryotic microalgae produce a wide range of biologically active compounds. These compounds include toxins, algaecides, plant-growth regulators and, most importantly, those with uses in the food and pharmaceutical industries (Arias, 2015).

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At the moment, both manufacturers and researchers are paying close attention to microalgae and cyanobacteria. These bacteria are anticipated to be significant commercial sources of valuable bioactive chemicals due to their very bio diverse characteristics. Specifically, the antibacterial qualities of cyanobacteria and microalgae have been investigated for their possible application as food preservatives and medicinal antimicrobial substances (Jena, 2018).

The investigation of these microbes for pharmaceutical use has produced significant chemical-based prototypes for the development of novel drugs, encouraging the application of advanced physical methods and novel biomedical compound synthesis. Furthermore, algae hold promise as sources of both new physiologically active chemicals and vital nutrients for human use (Schwartsmann, 2001). As a result, the economic sector requires a growing supply of algal extracts, fractions, or pure chemicals. Both primary and secondary metabolisms have been investigated in this context as a precursor to future logical economic exploitation.

Since many eukaryotic microalgae are historically employed as food and feed. Phenolics, carotenoids, flavonoids, lipids, alkaloids, and other bioactive substances with pharmacological significance can be found in abundance in marine microalgae. The short time required for generation, metabolic adaptability, lack of competition for arable land, ability to grow in any season, and little need for particular nutrients are some of the benefits of employing microalgae in drug discovery (Cardozo, 2007).

Compared to terrestrial plants, microalgae are richer in phytochemicals and bioactivity. For instance, microalgae are able to synthesize more carotenoids than higher plants, including lutein and astaxanthin. Furthermore, compared to higher plants, these carotenoids may be obtained more cheaply and with less effort. Both macroalgae and microalgae are abundant in flavonoids (Gröniger, 2000).

2. Bioactive Compounds in Microalgae

Microalgae are a valuable source of bioactive chemicals utilized in the food, medicinal products cosmetic, cosmeceutical, nutraceutical, and biomedicine industries. Numerous substances that have antibacterial, antiviral, or anti parasitic properties work well (Ferdous, 2021).

2.1. Derived Oligosaccharides and Polysaccharides

Polysaccharides, which can serve both structural and storage purposes, are typically the primary constituents of green, brown, and microalgae. Numerous polysaccharides, such as alginic acids and alginates, carrageenan and agar, laminarins, fucoidans, ulvans, and derivatives, make up the cell walls of algae (Balboa, 2013).

Their dispersion, size, concentration of charges, sulfate level (in sulfur-containing sugars), and structural and conformational features are some of the variables that affect their antibacterial action. Furthermore, oligosaccharides produced by depolymerizing algal polysaccharides provide plants with defense against bacterial, fungal, and viral infections (Vera, 2011).

Typically, several monosaccharides connected by diverse glucosidal linkages make up these polymeric carbohydrate frameworks. Disaccharide repeating units are found in the linear backbones of certain algal sugars, such as the ulvans of green algae and the sulphated galectins of red algae. Alginic acids are otherwise linear molecules composed of various groups of two monomeric units. Algal macromolecules consist of sulfated polysaccharides, which include cellulose and ulvan compounds from green algae, carrageenan and agar from red algae, and alginate, fucan, as well as laminarian from brown algae (Pérez, 2016).

2.2. Fatty Acids, Lipids, and Sterols

Since their biological role with particular conditions that are common in Western society, including obesity and cardiovascular diseases, has been clarified, fatty acid chains with more than two methylene-interrupted double bonds—which are necessary for normal cell function—have put the biomedical and nutraceutical fields. Furthermore, unsaturated lipids (PUFAs) are essential for tissue and cellular metabolism, including membrane control (Kumari, 2013).

Fatty acids are substances that are carboxylic acids that can be linear or branched and saturated or unsaturated, and have aliphatic chains with common even carbon numbers (C4–C28). Fatty acids are categorized as either monounsaturated or unsaturated fats based on the double bond. The final one may be categorized as n-3 or n-6 based on where the initial double bond is located from the methyl end. Red algae are high in C20 PUFAs, mostly

eicosapentaenoic (C20:5 n-3) arachidonic (C's20:4 n-6), while brown algae have both. Green algae are rich in C18 PUFAs, primarily α-linolenic (C's18:3 n-3), stearidonic (C18:4 n-3), and linoleic (C18:2 n-6) acids (Shilpi Gupta, 2011).

The percentage of algal lipids in microalgae varies between 0.12% to 6.73% (dry weight), and they are primarily made up of non-polar glycerolipids (neutral lipids), glycolipids, and phospholipids. The most common neutral lipid is triacylglycerol, which has a level that varies from 1% to 97% depending on retention and energy reservoir.

Phospholipids make up 10% to 20% of all lipids in algae and are found in extra-chloroplast membranes. The main fatty acids found in them are oleic, palmitic, stearic, arachidonic, and eicosapentanoic acids. They are distinguished by having larger concentrations of n-6 fatty acids. Green algae's phosphatidylglycerol, red algae's phosphatidylcholine, and brown algae's phosphatidylcholine and phosphatidylethanolamine are the three main phospholipids found in algae. Over fifty per cent of the lipids in the major algal groupings are glycolipids, which are found in photosynthetic membranes. High levels of n-3 unsaturated fatty acids define them. Sulfoquinovosyl diacylglycerides, monogalactosyl diacylglycerides, digalactosyl diacylglycerides are the three main forms of glycolipids (Ahmmed, 2020) .

Among the most significant chemical components of algal and a key dietary component for creatures raised in aquaculture are sterols. Many hydrobionts, particularly bivalves, eat microalgae as a key part of their diet. Although it varies by species, bivalves' capacity to bio converts or synthesis sterols from scratch is typically minimal and occasionally nonexistent. This suggests that bivalve organisms require a dietary source of sterol (C.R.K. Reddy, 2013).

2.3. Phenolic Substances

Since phenolic compounds do not directly participate in primary activities like photosynthesis, cell division, or algal reproduction, they are classified as secondary metabolites. An aromatic group with several hydroxyl groups is distinguishes by their antimicrobial action results from changes in microbial cell permeability, the destruction of internal macromolecules, or interference with membrane function, cellular integrity loss, and ultimately cell death (N. Abu-Ghannam, 2013).

2.4. Flavonoids

Algae are abundant in flavonoids, and by using various enhancement or manipulation methods, the level of flavonoid can be further raised. For instance, the buildup of flavonoids and phenolics is significantly impacted by elevated salt content. When exposed to a high concentration of Na Cl, the cyanobacterial types *Plectonema boryanum*, *Anabaena doliolum*, and *Oscillatoria acuta* grew less, while rutin accumulation increased (Brodowska, 2017). The buildup of flavonoids in algae can also be facilitated by metal stress. According to reports, *Dunaliella tertiolect* has higher levels of epicatechin and catechin along with more copper. These flavonoids are believed to protect microalgae cells from toxic effects of metals. These flavonoid-containing extracts also demonstrated antioxidant action (Campos, 2020).

Increased levels of nitrate and the growth nutrient L-phenylalanine caused Spirulina maxima to accumulate more flavonoids, including kaempferol and quercetin. In rats, the lipid-peroxidation of hepatic microsomes, which caused by the oxidizing agent carbon tetrachloride was prevented by the existence of these flavonoids and other phenolics. The outcomes were similar to those of the brand-name antioxidant (Kopustinskiene, 2020).

2.5. Pigments

As photosynthetic creatures, algae are able to synthesize the three primary classes of pigments present in marine algae: phycobiliproteins, carotenoids, and chlorophylls. This enables seaweed to be categorized into three groups: Rhodophyceae (red algae), Phaeophyceae (brown algae), and Chlorophyceae (green algae). The high content of chlorophylls a and b gives the color green, fucoxanthin and chlorophylls a and c give it a greenish brown hue, and phycobilin such phycoerythrin and phycocyanin give it a red hue (S Pinteus, 2015).

Lysozyme, an immunological enzyme that breaks down bacterial cell walls, may accumulate as a result of the antibacterial mechanism for carotenoids that has been suggested. All algae include carotenoids, which are lipid-soluble, naturally occurring colors made up of eight tetraterpenoids, or units of five carbons, with up to fifteen conjugated double bonds. Carotenoids are often classified into two classes: xanthophyll or oxycarotenoids (that contain a minimum of one atom of oxygen, such as a hydroxyl group, an oxy-group, or a mix of both) and carotenes (where the chain ends with a cyclic group, having just carbon and hydrogen atoms). The most prevalent carotene is β-carotene, while the xanthophylls class includes lutein, fucoxanthin, and violaxanthin (Christaki, 2013).

2.6. Other substances

Other secondary metabolites, such as aromatic compounds, alkaloids, lectins, or halogenated chemicals, have variety of anti-fungal, antibacterial, antibacterial, antimacrofouling, and antiprotozoal properties can also be produced by microalgae.

2.6.1. Lectins

Natural bioactive ubiquitous proteins, also known as glycoproteins of non-immune response, lectins bind reversibly to polysaccharides, glycolipids, and glycans of glycoproteins that have at least one non-catalytic domain, resulting in agglutination. As they are a single-molecule, proteins of low molecular weight with a high concentration of acidic amino acids and an isoelectric point between 4 and 6, algae lectins are different from terrestrial lectins (Singh, 2013). These are monosaccharides, do not need ions of metal for their biological activities. Algal lectins are divided into three main groups according to their ability to bind to glycoproteins: complicated type N-glycan particular lectins, higher mannose kind N-glycan sensitive lectins, and lectins that are specific to both of the aforementioned N-glycan types. Additionally, lectins from marine species are divided into rhamnose-binding lectins, galectins, intelectins, F-type lectins, and C-type lectins (Cheung, 2015).

2.6.2. Terpenes

One of the main groups of metabolites that marine algae make are terpenes. They are generated from the five-carbon precursor isopentenyl pyrophosphate. While Rhodophyceae are distinguished by a great variety of structures of halogen-containing secondary metabolites, whose polyhalogenated monoterpenes demonstrate a broad spectrum of antibacterial properties, (Bedoux, 2014). This family contains cyclic and straight sesqui-, di-, and triterpenes. Capisterones, that are triterpene sulfate esters, have strong antifungal properties, while many of them have antiviral properties. Others, like the sesquiterpene (−)-elatol, are antifouling agents (Gamal, 2010)

2.6.3. Alkaloids

A substance with one or more nitrogen atoms in a heterocyclic ring is called an alkaloid. Terrestrial plants did not contain them. Three categories of alkaloids were identified in marine algae: (i) triphenylamine (ii) halogen-containing indole alkaloid; and (iii) additional alkaloids, including derivatives of 2,7-naphthyridine. The majority of the alkaloids that have been extracted from marine algae are indole and 2-phenylethylamine families (Güven, 2010,). Halogenated alkaloids are unique to algae; they are alkaloids that contain bromine and chloride, which are especially prevalent in Chlorophyta. Rhodophyta contains the majority of the indole group's alkaloids (Barbosa, 2014).

3. Experimental method

3.1. Materials

The algal extracts were prepared using a variety of organic solvents, including solutions of ethanol, benzene, ethyl acetate, diethyl ether, chloroform, hexane, and methanol. These samples were then submitted to chemical analysis and utilized for antimicrobial test screening. Antimicrobial studies against the hazardous microbes, *Bacillus subtilis*, *Pseudomonas S*. *typhimurium*, *Klebsiella pneumonia*, *Vibreo cholerae*, *Staphylococcus aureus*, *bacillus Candida* albicans, *A. niger,* and *Aspergillus flavus* were conducted using the extracted carotenoid pigments and chlorophylls.

3.2. Growing Conditions for Microalgal Strains

The antibacterial activity of the microalgae was checked in *Scenedesmus sp., Isochrysis galbana*, *Chlorella sp*. *Nannochloropsis*, *Chlorella vulgaris*, *Haematococcus pluvialis*, *Dunaliella tertiolecta*, *Tetraselmis astigmatica*, *Tetraselmis chuii,* and *Tetraselmis suecica*.

Plankton nets were used to gather microalgae samples from the sea water and transport them to a lab. The study employed both gram-positive and gram-negative harmful microorganisms. E. coli and *P. aeruginosa* were the gram-positive bacteria employed in the antimicrobial investigation, while *S. aureus* and *B. subtilis* were the gram-negative bacteria.

In order to get isolated colonies, serial dilution was used. The temperature was kept at room temperature, 25 °C ± 2 °C, and the medium's pH was adjusted to 7.5. The fluorescence microscope was used to see and identify the material. In 2 L conical flasks, microalgae were sub-cultured from master cultures using Bold's basal medium (BBM) for fresh microalgae and f/2 media for marine microalgae. In accordance with Alsenani et al. (2019), the various microalgal

species were cultivated. Once the OD (optical density) at 440 nm reached 2.5, cultures were moved to 20 L bioreactors in under the same circumstances after the development of the algae came to the late exponential phase. With a 12/12hour light cycle, the cultures were cultivated externally in a 2 m sealed photobioreactor made up of 200 L clear bag towers that had a 36 cm diameter. The temperature fluctuated within 23.6 °C at the day and 13.9 °C at night. Centrifugation at 500g for 2 minutes was used to collect the cultures, and Milli-Q water was used for washing. Two batches of each collected biomass were made; the first group was utilized straight away for antimicrobial tests at room temperature (25 °C ± 2 °C). Ten milliliters of BG 11 medium were used to cultivate the colony. To prepare it for mass culture, it was then moved into 250 ml of media and corrected to 500 ml. Prior to the extraction procedure, the second group was freeze-dried and kept at -40 °C. To test for extracellular antibacterial activity, the supernatants were also collected and kept at -20°C. Cyanobacteria were grown in BG-11 media in a 2-L Erlenmeyer's flask and incubated in racks that were illuminated by fluorescent lights. The LI-250 light meter was used to measure the light intensities during the 12-hour dark period of the photo-incubation, which was conducted at 25°C. To achieve uniform growth, the disinfected inoculated loop was immersed in the bacterial solution then the loopful of solution distributed over the nutritional agar surface. The agar's rim was swept one last time. For eighteen hours, the culture was cultivated at 37 °C.

3.3. Extraction Techniques

The bioactive compounds were screened using a variety of extraction techniques and systems. Ethyl acetate, methanol, dichloromethane, n-hexane chloroform, dimethyl sulfoxide, water, chloroform+ methanol (2:1, v/v), and water + methanol (1:3, v/v) were among the solvent systems that were employed.

- Approach (a): In a porcelain mortar, 10 mg of biomass from algae (dry weight (DW)) was combined with 1 mL of each solvent, and the mixture was then crushed. After that, the extracts were put into 2 mL tubes and allowed to shake at room temperature for 90 minutes. After centrifuging the tubes at 5000 g for one minute, the intracellular extracts, or supernatants, were collected and moved to a second set of 2 mL tubes.
- Method (b): To stop light-sensitive chemicals from degrading, 20 mL of each liquid was introduced to 1g of the algae biomass, vortexed for 30 s, and then incubated in a dark environment for the whole night. Following centrifugation to the extracts were moved to fresh tubes, and rotary evaporation was used to thoroughly remove the solvents. 200 µL of the chosen solvents were then used to re-dissolve the crude extracts.

A four-solvent system comprising of ethyl acetate, water + methanol (1:3, v/v) chloroform, and acetone were used in sequence, for the following extraction in method (c). Every extraction stage required an overnight dark incubation period.

Rotating evaporation was used to remove the extract from each solvent. 100 µL of an identical solvent solution was used to resuspend the dried extracts. Every extract was kept at -20°C and shielded from sunlight at all times.

Hexane, dichloromethane, and methanol were used to separate the active crude extracts. Following the completion of antimicrobial testing for every fraction, the most active fractions were extracted for further examination.

3.4. Antimicrobial Test

The antibacterial activity of the chosen microalgae and cyanobacteria was tested in both extracellular and intracellular extracts. To guarantee the correctness of the findings, the disc-diffusion approach was applied. Initially, tryptone soy agar (TSA) was poured onto the plates using the disc-diffusion method, and the plates were left to solidify for 20 minutes. After that, the medium was distributed over the stock cultures to inoculate them. Using sterile forceps, discs were put over the TSA after being filled with 50 µL of each microalgal extract and left to dry. An inhibitory zone, a marker of antimicrobial action, was seen during an overnight incubation at either 25 °C or 37 °C, depending on the bacterium. The plates were then spotted with 20 µL of different microalgal extracts, and they were left to dry for forty-five minutes. Inhibition zones were seen following an overnight incubation period.

3.5. Minimum Inhibition Concentration

A soy agar tryptic plate was streaked with the bacteria to be examined, and it was then incubated for 24 hours at either 28 or 37 degrees Celsius. The cell density was then increased to 105-110 colony-forming cells (cfu)/mL after one colony was moved to 15 mL of fresh tryptic soy broth. The test extracts were diluted with 10% ethanol using sterile deionized water after being dissolved in 100% ethanol. Freshly made microbiological broth was placed to each well of the microtiter plate with 96 wells after each dilution had been moved there. Positive growth controls included both growth medium and bacterial suspension, whereas negative growth controls solely included growth media. Each well's optical density was determined spectrophotometrically at 600 nm after the plates were incubated for 24 hours at 37 °C. The

smallest quantity of each solution being tested that prevented the development of every microbe in the wells was recorded as the MIC value, which was established during an overnight incubation period. For every sample, the tests were carried out in triplicate, and the mean quantity of each triple was determined. The minimum amount of the extracts in the medium of broth that prevented the test microorganism's apparent growth was identified as the minimal inhibitory concentration value.

3.6. Gas-Chromatography with Mass Spectrometer Analysis

Gas Chromatography-Mass Spectroscopy was used to quantify and identify the bioactive chemicals that were found in the liquid extracts of the microalgal species and that were crucial to the antibacterial activity. The chromatographic column was an Agilent Technologies model 7890A (GC system) type, with an HP-5 column (30 m × 0.25 mm ID × 0.25 µm film thickness). The following were the chromatographic conditions: Helium carrier gas was used to inject one microliter of algal extract into the GC-MS at a flow rate of two milliliters per minute, with the injectors and column oven temperatures set to 280 × 70 °C and the injection mode set to "split" with a split ratio of 20:1. After one minute of maintaining an isothermal oven temperature at 80 °C, the temperature was raised to 400 degrees Celsius at an average rate of 4 °C per minute and maintained there for five minutes. Conditions for MS (Agilent Techniques 5975C model) were as follows: 200 °C for the ion source, 300 °C for the interface, and 40–1000 mass units for the mass range.

3.7. High Performance Liquid Chromatography

An analytical server and a Shimadzu, Inc Nexera-i LC-2040C 3D analyzing high-performance liquid chromatographic system with a photo-diode array detectors (200–800 nm), a column with a temperature of 22 °C, a web-based degasser, an autosampler, and a quaternary solutions delivery system were used to examine each active fraction. The HPLC system was filled with 10 µL of portions of the bioactive fractions that had been redissolved in methanol. On a Jupiter C4 microbore column, chromatographic separation was carried out using gradient elution with 0.1 percent formic acid (A) and acetonitrile (B). The gradient for 110 minutes was as follows: 100% phase A for 0 minutes, 100% phase B for 90 minutes, and 100% phase B for 110 minutes. 0.2 mL/min was the flow rate. Prior to injection, the samples were filtered using a 0.2 µm polytetrafluoroethylene, or PTFE membrane filter (Bose, 2015).

4. Result

Three species *I. galbana*, *C. muelleri*, and *T. suecica* of the several microalgae and cyanobacteria that were analyzed shown efficacy against gram-positive infections on plate growth inhibition studies. The antibacterial efficacy of various solvent extracts against certain infections varied. When employing a disc-diffusion experiment, the ethanolic extract of *I. galbana* exhibited the highest degree of antibacterial action against L. monocytogenes, whereas the ethyl acetate extract showed less activity. Additionally, the spot-on-lawn approach demonstrated a minor growth suppression efficacy of *I. galbana's* ethanol extract against *S. aureus*. Nevertheless, when employing the disc-diffusion approach, no other extracts shown any activity.

The spot-on-lawn approach revealed that different extracts had greater growth inhibitory activity. All gram-positive bacteria were significantly inhibited by extracts of the three microalgal species (Fig. 1). However, none of the extracts showed any antibacterial action against gram-negative bacteria.

4.1. Minimum inhibition concentration.

Multiple dilutions of with a maximum of 100 µg of extracts/mL of 1% DMSO were used to determine the extracts' MICs. The microalgae under test had active growth inhibition doses that varied between 500 µg/mL to 1 mg/mL. I. galbana had a MIC of 500 µg/mL, while *T. suecica*, FN1, and *C. muelleri* had MICs of 1 mg/mL. Below the specified doses of 100 µg/mL, no indication of growth suppression was seen. The lowest doses that produced clear solutions and prevented all bacterial growth were found and tested.

Table 1 Summary of antimicrobial activities of various species. Shown are mean values ± SD from three separately cultures

Species	**Empty Cell**	***I. galbana***	**Empty Cell**	***T. suecica***	***C. muelleri***
Extracts		100% ethyl acetate	100% ethanol	70% methanol in water	70% methanol in water
Fractions			Hexane	Hexane	Hexane

Target pathogens	*L. monocytogenes*	11.67[a](±0.58)	18.67 (±0.58)*	15.67(±0.58)*	17.3(±1.15)*
	S. aureus	–	20(0)*	15.53(±0.58)*	16.68(±1.53)*
	B. subtilis	–	18.33(±0.58)*	14.67(±1.15)*	17.83(±1.15)*
	E. coli	–	–	–	–
	S. typhoid	–	–	–	–
	P. syringae	–	–	–	–
	C. michiganensis	–	16.33(±2.31)*	18.67(±1.53)*	17(0)*
	S. epidermidis	–	18.55(±0.58)*	17.23(±0.58)*	16.67(±0.58)*
	E. faecalis	–	19.33(±1.15)*	19.67(±0.58)*	18.67(±1.15)*

(–) No zone of inhibitions were observed.

4.2. GC-MS examination

The extracts were characterized using GC–MS in an effort to pinpoint the substances that gave the microalgae their antibacterial properties. Different species' fatty acid methyl esters (FAME) concentrations and profiles were displayed by the GC-MS data. As a FAME percentage, *T. suecica* FN1 had the greatest levels of α-linolenic acid (68.3%), *I. galbana* had the highest levels of oleic acid (35.85%), and *T. suecica* FN1 had the highest levels of linoleic acid (12.09%). Furthermore, all three species had varying amounts of hexadecenoic acid, linoleic acidoleic acid, and α-linolenic acid, but only I. galbana had EPA (eicosapentaenoic acid) and docosahexaenoic acid (DHA) (0.86% and 24.61%, respectively).

Table 2 GC–MS identification of fatty acids methyl esters (FAME) detected in microalgal species, showing mean values ± SEs as mg/g dry weight from three separately grown cultures (n = 3)

Fatty acids		***I. galbana***	***C. mulleri*. NT8c**	***T. suecica*. FN1**
Palmitic acid	C16:0	24.73 ± 1.07	20.82 ± 0.76	24.60 ± 1.02
Hexadecenoic acid	C16:1	10.64 ± 0.67	7.48 ± 0.34	7.16 ± 0.21
Octadecanoic acid	C18:0	0.76 ± 0.06	0.53 ± 0.04	0.58 ± 0.08
Oleic acid	C18:1	36.80 ± 2.12	5.25 ± 0.3	11.93 ± 0.59
Linoleic acid	C18:2n6	4.58 ± 0.04	7.36 ± 0.22	28.54 ± 0.926
α-Linolenic acid	C18:3n3	6.53 ± 0.46	25.57 ± 0.57	158.54 ± 3.388
EPA	C20:5n5	0.98 ± 0.13	n.d.	n.d.

5. Discussion

The antibacterial properties of cyanobacteria and microalgae have been the subject of several investigations. Chlorellin comes from Chlorella sp. and was the first antibacterial chemical found in microalgae (Gunnison, 1997). Furthermore, it has been determined that phycobiliproteins, fatty acids, carotenoids, and derivatives of chlorophyll are important bioactive substances found in cyanobacteria and microalgae assessed *I. galbana's* antibacterial activity and discovered that it strongly inhibited bacteria and fungus, with a MIC ranging from 2.6 to 4.3 mg/mL and activity associated with the presence of fatty acids and carotenoids (Maadane, 2017).

The percent of n-hexane Gram-positive bacterial growth was similarly suppressed by *I. galbana*. Additionally, it has been stated that the lipids from *Nanochloropsis oculata* demonstrated activity against both gram-positive and gram-negative bacteria, and that the both short- and lengthy-chain fatty acids from *H. pluvialis*, and *C. muelleri* are in charge of growth restriction against *B. subtilis*, *S. aureus*, and *E. coli*. Additionally, *I. galbana* demonstrated a robust suppression of a broad spectrum of gram-negative bacteria and a moderate inhibition of *S. aureus*, indicating antibacterial action induced by a substance called pheophytin A and chlorophyllide A (Ustinskaya, 2019).

The minimum inhibitory concentration (MIC), which is the smallest quantity of extract that totally stops the spread of the bacteria in comparison to other extracts, was used to determine the activity's intensity. Furthermore, it was asserted that the growth inhibitory action brought on by the harmful effects of fatty acids results in bacterial cell membrane leakage or disturbances in nutrient absorption. The composition of the cell wall distinguishes gram-positive bacteria from gram-negative bacteria. Gram-negative organisms have an envelope-like outer membrane (Srinivasakumar, 2009). The cytoplasmic membrane, also known as the inner layer of the cell wall made of peptidoglycan, and the outer membrane make up this multilayered structure. Certain substances, such as antibiotics, are prevented from entering the cell by this outer membrane, which acts as an obstruction to permeable substances. Gram-positive bacteria were less susceptible to antibiotics and more vulnerable than gram-negative bacteria, which can be explained by this outer cell wall function (You-Jin, 2001).

6. Conclusion

In addition, cyanobacteria and microalgae have demonstrated antiviral and antifungal properties against a variety of microbes. Chlorella vulgaris, Scenedesmus, obliquus, *Chlamydomonas reinhardtii* and *Oocystic sp*. extracts in methanol and hexane inhibit the growth of *Aspergillus fumigatus, Candida kefyr, and Aspergillus niger*. However, a number of investigations have discovered substances in microalgae that limit the growth of viruses.

This study demonstrates that microalgae have the potential to generate potent antimicrobial compounds that act against human and plant pathogens when extraction processes are performed using appropriate solvents and conditions. The study found that three species of microalgae can produce strong antimicrobial compounds that inhibit the growth of different species of pathogenic gram-positive bacteria. The inhibition activity was caused by unsaturated fatty acids and carotenoids – compounds that were extracted efficiently from the selected species. These bacteriostatic or bactericidal bioactive compounds may act independently or through synergistic actions, although their precise mechanism of action is still unknown.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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